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Effect of diet composition on coenzyme A and its thioester pools in various rat tissues

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ABSTRACT

Three coenzyme A (CoA) molecular species, *i.e.*, acetyl-CoA, malonyl-CoA, and nonesterified CoA (CoASH), in 13 types of fasted rat tissue were analyzed. A relatively larger pool size of total CoA, consisting of acetyl-CoA, malonyl-CoA, and CoASH, was observed in the medulla oblongata, liver, heart, and brown adipose tissue. Focusing on changes in the CoA pool size in response to the nutrient composition of the diet given, total CoA pools in rats continuously fed a high-fat diet for 4 weeks were significantly higher in the hypothalamus, cerebellum, and kidney, and significantly lower in the liver and skeletal muscle than those of rats fed a high-carbohydrate or high-protein diet. In particular, reductions in the liver were remarkable and were caused by decreased CoASH levels. Consequently, the total CoA pool size was reduced by approximately one-fifth of the hepatic contents of rats fed the other diets. In the hypothalamus, which monitors energy balance, all three CoA molecular species measured were at higher levels when rats were fed the high-fat diet. Thus, it was of interest that feeding rats a high-fat diet affected the behaviors of CoA pools in the hypothalamus, liver, and skeletal muscle, suggesting a significant relationship between CoA pools, especially malonyl-CoA and/or CoASH pools, and lipid metabolism *in vivo*.

1. Introduction

Coenzyme A (CoA) is synthesized from a water soluble vitamin, pantothenate, through five enzymatic steps [1,2]. Since the essential cofactor is utilized as a carrier of the acyl group by approximately 4% of all enzymes [3], a nonesterified CoA (CoASH) or its thioesters appear in various pathways. However, the intracellular behaviors of CoASH and acyl-CoAs are not well known because the methods for determining these intracellular compounds with sufficient sensitivity have not been established. We developed an enzymatic method, the acyl-CoA cycling method [4–6]. This method can detect the *in vivo* major CoA species of acetyl-CoA, malonyl-CoA, and CoASH to pmol levels, and it has revealed changes in the size and composition of CoASH and acyl-CoAs in bacterial cells [7–9]. Recently, analysis using the acyl-CoA cycling method was extended to mammals and the following results were obtained: (i) the role of malonyl-CoA as a regulator of food intake in hypothalamus [10–16], (ii) the contribution of acetyl-CoA generated from acetate to thermogenesis during fasting [16], and (iii) the enhancement of lipid degradation in cancer cachexia by decreased malonyl-CoA levels [17]. In the analysis of various tissues from rats, it has been elucidated that malonyl-CoA levels, not only in the hypothalamus but also in other brain tissues, increase in response to food intake and the liver, heart, and brown adipose tissue possess larger CoA pools than those of other tissues measured [18].

Here we describe a comprehensive analysis of CoA pools in rat tissues responding to diets with a high-carbohydrate, protein, or fat content. Intracellular CoA pools were predicted to keep a constant size, since the eukaryotic CoA biosynthetic pathway is strictly regulated by pantothenate kinases, which catalyze the committed step of the phosphorylation of pantothenate [19–21]. However, the size and composition of CoA pools consisting of acetyl-CoA, malonyl-CoA, and CoASH were different depending on the kind of tissue, and pool sizes in the hypothalamus, cerebellum, medulla oblongata, liver, kidney, and skeletal muscle also changed according to differences in the nutrient composition of the fed diets.

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2. Materials and methods

2.1. Handling of rats and extraction of CoA pools

Animal experiments were conducted in accordance with guidelines of the Ibaraki University Animal Research Committee. Eight-week-old male Wistar rats (250–275 g) were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Rats were fed standard laboratory chow (MF from Oriental Yeast Co. Ltd., Tokyo, Japan) and given water *ad libitum* under a 12-h light (6:00 am–6:00 pm)/12-h dark (6:00 pm–6:00 am) cycle at 22 ± 1 °C in individual cages. After a week, rats were divided into three groups, were composed of six individuals, and were fed a high-carbohydrate diet (HCD), which was the same as AIN-93G, a high-protein diet (HPD), or a high-fat diet (HFD) prepared in Oriental Yeast Co. Ltd. for 4 weeks (Table 1). These diets were composed of the following nutrients in a caloric ratio: HCD, 63.5:18.8:17.7 (carbohydrate:protein:fat); HPD, 25.6:56.5:17.9; HFD, 19.6:18.2:62.2. Rats were starved for 16 h, and then 13 types of tissue, *i.e.*, cerebral cortex, hippocampus, hypothalamus, cerebellum, medulla oblongata, liver, spleen, kidney (right side), heart, skeletal muscle from the right soleus muscle, perirenal adipose tissue, brown adipose tissue, and epididymal adipose tissue, were removed. The CoA pools in these tissues were extracted by homogenization in 400 μl of 0.6 M sulfuric acid per each 100 mg of tissue, and then the homogenized tissues were kept at 4 °C overnight for complete extraction of the intracellular CoASH and its thioesters and inactivation of endogenous enzymes. After centrifugation at 9000*g* at 4 °C for 10 min, 1 M Tris (0.05 vol. of the supernatant) was added to the supernatant and the resulting solution was carefully adjusted to around pH 6.5 with NaOH on ice. The neutralized solution was kept at −80 °C overnight and the clear extract was recovered by centrifugation after thawing. Acetyl-CoA, malonyl-CoA, and CoASH in the extract were stable for at least a week at 4 °C.

Table 1
Composition of the diets used in this study.

	HCD (g/100 g diet)	HPD (g/100 g diet)	HFD (g/100 g diet)
Milk casein	20.0	59.7	25.6
L-Cystine	0.30	0.92	0.36
Maltodextrin	–	–	6.0
Cornstarch	39.7	–	–
Pregelatinized cornstarch	13.2	12.6	16.0
Sucrose	10.0	10.0	5.5
Soybean oil	7.0	7.0	2.0
Lard	–	–	33.0
Cellulose powder	5.0	5.0	6.61
AIN-93G mineral mix[a]	3.5	3.5	3.5
AIN-93 vitamin mix[b]	1.0	1.0	1.0
Choline bitartrate	0.25	0.25	0.25
Calcium carbonate	–	–	0.18
Tert-butyl hydroquinone	0.0014	0.0014	–

[a] Minerals in 100 g diet: $CaCO_3$, 1250 mg; KH_2PO_4, 686 mg; $K_3C_6H_5O_7{\cdot}H_2O$, 248 mg; NaCl, 259 mg; K_2SO_4, 163 mg; MgO, 84 mg; $FeC_6H_5O_7{\cdot}nH_2O$, 21 mg; $ZnCO_3$, 5.8 mg; $MnCO_3$, 2.2 mg; $CuCO_3{\cdot}Cu(OH)_2{\cdot}H_2O$, 1.1 mg; Na_2SeO_4, 0.036 mg; $(NH_4)Mo_7O_{24}{\cdot}4H_2O$, 0.028 mg; $NaSiO_3{\cdot}9H_2O$, 5.1 mg; $CrK(SO_4)_2{\cdot}12H_2O$, 0.96 mg; LiCl, 0.061 mg; H_3BO_3, 0.29 mg; NaF, 0.22 mg; $NiCO_3{\cdot}2Ni(OH)_2{\cdot}4H_2O$, 0.11 mg; NH_4VO_3, 0.023 mg.

[b] Vitamins in 100 g diet: all-trans-retinyl acetate, 400 IU; cholecalciferol, 100 IU; all-rac-α-tocopherol acetate, 7.5 mg; phylloquinone, 75 μg; thiamine hydrochloride, 0.6 mg; riboflavin, 0.6 mg; pyridoxine hydrochloride, 0.7 mg; cyanocobalamin, 2.5 μg; biotin, 20 μg; folic acid, 0.2 mg; calcium pantothenate, 1.6 mg; nicotinic acid, 3 mg.

2.2. Measurement of CoASH, acetyl-CoA, and malonyl-CoA in the tissue extract

CoASH, acetyl-CoA, and malonyl-CoA in the extracts were measured by the acyl-CoA cycling method using malonate decarboxylase [4–6,18]. The enzyme was purified from *Pseudomonas putida* JCM 20089 (formerly IAM 1177) as previously described [22]. The reaction mixture of the acyl-CoA cycling method contained 50 mM Tris–HCl (pH 7.2), 1 mM 2-mercaptoethanol, 10 mM $MgSO_4$, 50 mM malonate, 10 mM ATP, 1 U of malonate decarboxylase, and the tissue extracts containing acetyl-CoA and/or malonyl-CoA (2.5–80 pmol) in 400 μl. The cycling reaction was initiated by the addition of malonate decarboxylase, and the mixture was incubated at 30 °C for 20 min, followed by the addition of 1 U of acetate kinase from *Escherichia coli* ([EC 2.7.2.1], Roche Diagnostics GmbH, Mannheim, Germany). After 20 min of incubation, 0.2 ml of 2.5 M neutralized hydroxylamine was added, and the incubation was continued for an additional 20 min at 30 °C. The reaction was terminated by adding 0.6 ml of 10 mM ferric chloride dissolved in 25 mM trichloroacetic acid-1 M HCl. The A_{540} of the acetohydroxamate formed was measured. Every assay was performed in duplicate. Separate determinations of malonyl-CoA and CoASH in the tissue extracts were accomplished by eliminating acetyl-CoA with citrate synthase (EC 4.1.3.7) and by converting CoASH to acetyl-CoA with phosphate acetyltransferase (EC 2.3.1.8), respectively, before the measurements using the acyl-CoA cycling method. The reaction mixture for the citrate synthase treatment contained 50 mM Tris–HCl (pH 7.2), 10 mM $MgSO_4$, 2 mM oxaloacetate, 1 U of citrate synthase from porcine heart (Roche Diagnostics GmbH), and the tissue extracts in 1 ml. The reaction was carried out at 25 °C for 20 min and terminated by placing the reaction tube on ice slush. The reaction mixture for CoASH measurement contained 50 mM Tris–HCl (pH 7.2), 1 mM 2-mercaptoethanol, 10 mM $MgSO_4$, 10 mM ammonium sulfate, 0.1 mM acetylphosphate, 5 U of phosphate acetyltransferase from *Bacillus stearothermophilus* (Sigma–Aldrich Inc., St. Louis, MO, USA), and the tissue extracts in 1 ml. After incubation at 25 °C for 20 min, the reaction was terminated by filtration through a Millipore Ultracel YM-30 ultrafilter to eliminate the phosphate acetyltransferase.

2.3. Biochemical analysis of blood serum

Glucose (Glc), triglycerides (TGs), nonesterified fatty acids (NEFAs), and total ketone bodies (T-KB) in the serum were analyzed in Nagahama Life Science Laboratory (Shiga, Japan).

2.4. Statistical analysis

Statistical significance was assessed by the one-way ANOVA with Tukey's multiple comparison tests using Excel Toukei 2010 for Windows (Social Survey Research Information Co. Ltd., Tokyo, Japan).

3. Results and discussion

3.1. Body weight gain, tissue weight, and blood serum parameters

Rats were fed on three different diets, HCD, HPD, or HFD, for 4 weeks. As shown in Table 2, there were no significant differences in energy intakes between HCD-, HPD-, and HFD-fed rats. However, body weight gain in HFD-fed rats was significantly larger than that of HCD- or HPD-fed rats. Continuous intake of HFD for 4 weeks led to diet-induced obesity and significantly heavier tissue weights were observed in the liver, heart, and epididymal adipose tissue. Inversely, the kidney was lighter. In perirenal adipose tissue,

Table 2
Energy intake, body weight gain, and tissue weights in rats.

	HCD	HPD	HFD
Energy intake (kcal/day)	107 ± 5	99 ± 8	105 ± 4
Body weight gain (g)	112 ± 8[a]	90 ± 6[a]	156 ± 7[b]
Liver (g)	11.1 ± 0.7[a]	10.4 ± 0.3[a]	14.4 ± 0.5[b]
Spleen (g)	0.97 ± 0.08	0.98 ± 0.05	0.94 ± 0.08
Kidney (g)	1.49 ± 0.08[a]	1.63 ± 0.03[a]	1.30 ± 0.03[b]
Heart (g)	1.11 ± 0.02[a]	1.05 ± 0.02[a]	1.24 ± 0.03[b]
Skeletal muscle (g)	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
Perirenal adipose tissue (g)	9.07 ± 1.56[a,b]	5.68 ± 0.61[a]	12.5 ± 1.0[b]
Brown adipose tissue (g)	0.83 ± 0.07[a]	0.43 ± 0.04[b]	1.06 ± 0.09[a]
Epididymal adipose tissue (g)	8.61 ± 0.69[a]	6.77 ± 0.42[a]	12.4 ± 0.8[b]

All data are expressed as means ± SEM ($n = 6$).
[a,b]Significance was analyzed by the one-way AVOVA with Tukey's multiple comparison tests. Mean values with different letters were significantly different ($P < 0.05$).

Table 3
Analysis of glucose, triglycerides, nonesterified fatty acids, and total ketone bodies in blood serum.

	HCD	HPD	HFD
Glucose (mg/dL)	183 ± 15	198 ± 10	173 ± 11
Triglycerides (mg/dL)	44.7 ± 6.0	36.4 ± 4.5	28.5 ± 2.5
NEFAs (μEq/L)	315 ± 33	304 ± 44	314 ± 38
T-KB (μmol/L)	1221 ± 185	1110 ± 183	1218 ± 138

All data are expressed as means ± SEM ($n = 6$).

although there were no significant differences between HCD- and HPD-fed rats and HCD- and HFD-fed rats, a significant difference existed between HPD- and HFD-fed rats. In HPD-fed rats, brown adipose tissue was lighter than tissues from rats belonging to the two other groups. Thus, although body weight gain and tissue weight were affected by the components of the diets, no significant differences between the three groups were detected in Glc, NEFAs, TGs, and T-KB levels in sera using the one-way AVOVA with Tukey's multiple comparison tests (Table 3).

3.2. CoA pools in the rat tissues

The effects of the three different diets on the levels of three CoA molecular species, *i.e.*, acetyl-CoA, malonyl-CoA, and nonesterified CoA (CoASH), in rat tissues were comprehensively analyzed using the acyl-CoA cycling method (Table 4). In brain tissues, acetyl-CoA and malonyl-CoA levels changed within the narrow range of 1.75–3.87 and 0.209–0.441 nmol/g of tissue, respectively. On the other hand, CoASH levels broadly varied from 0.600 to 7.00 nmol/g of tissue, and the medulla oblongata formed the largest CoASH pool among the five brain tissues regardless of the diets given. The sizes of total CoA pools, which indicate the sum of acetyl-CoA, malonyl-CoA, and CoASH, in brain tissues were within 2.61 to 9.62 nmol/g of tissue. Significant differences in total CoA pool sizes were detected in the hypothalamus, cerebellum, and medulla oblongata. Interestingly, the hypothalamus, which controls food intake through malonyl-CoA as a mediator, formed a significantly larger total CoA pool by continuous feeding with HFD than that of rats fed HCD and HPD, and this phenomenon was found in all three CoA pools, *i.e.*, acetyl-CoA, malonyl-CoA, and CoASH pools. Augmentation of the total CoA pool by intake of the high-fat diet also occurred in the cerebellum. Inversely, the total CoA pool in the medulla oblongata became smaller in rats fed HFD, and there was a significant difference between HFD- and HCD-fed rats. Meanwhile, there were no significant differences in total CoA pool sizes of brain tissues from HCD- and HPD-fed rats.

Table 4
CoA pools in rat tissues.

Tissues	CoA species	HCD	HPD	HFD
Cerebral cortex	A	2.08 ± 0.08	2.34 ± 0.12	2.41 ± 0.13
	M	0.273 ± 0.014	0.242 ± 0.012	0.209 ± 0.033
	CoA	0.601 ± 0.324	1.08 ± 0.24	1.48 ± 0.36
	Total	2.95 ± 0.39	3.66 ± 0.19	4.09 ± 0.42
Hippocampus	A	2.00 ± 0.03[a]	2.45 ± 0.03[a,b]	2.75 ± 0.25[b]
	M	0.347 ± 0.019	0.323 ± 0.021	0.377 ± 0.055
	CoA	1.44 ± 0.34	0.694 ± 0.218	1.19 ± 0.29
	Total	3.78 ± 0.34	3.46 ± 0.24	4.31 ± 0.34
Hypothalamus	A	1.75 ± 0.08[a]	1.90 ± 0.19[a]	2.94 ± 0.25[b]
	M	0.258 ± 0.014[a]	0.270 ± 0.024[a]	0.358 ± 0.013[b]
	CoA	0.600 ± 0.188[a]	1.43 ± 0.65[a,b]	3.19 ± 0.94[b]
	Total	2.61 ± 0.27[a]	3.57 ± 0.57[a]	6.48 ± 0.78[b]
Cerebellum	A	3.32 ± 0.18[a]	3.51 ± 0.05[a,b]	3.87 ± 0.18[b]
	M	0.380 ± 0.022	0.381 ± 0.033	0.371 ± 0.028
	CoA	0.650 ± 0.230[a,b]	0.428 ± 0.137[a]	2.08 ± 0.75[b]
	Total	4.35 ± 0.29[a]	4.32 ± 0.18[a]	6.32 ± 0.71[b]
Medulla oblongata	A	2.23 ± 0.09	2.15 ± 0.16	2.60 ± 0.12
	M	0.397 ± 0.013	0.367 ± 0.023	0.441 ± 0.021
	CoA	7.00 ± 0.32[a]	6.76 ± 0.36[a]	4.78 ± 0.44[b]
	Total	9.62 ± 0.38[a]	9.27 ± 0.49[a,b]	7.82 ± 0.53[b]
Liver	A	5.01 ± 0.52	3.38 ± 0.57	5.03 ± 0.83
	M	2.06 ± 0.21[a]	1.86 ± 0.23[a]	0.560 ± 0.074[b]
	CoA	97.1 ± 4.6[a]	90.2 ± 3.2[a]	15.7 ± 2.5[b]
	Total	104 ± 5[a]	95.5 ± 3.3[a]	21.2 ± 3.1[b]
Spleen	A	1.22 ± 0.06	1.09 ± 0.07	1.23 ± 0.10
	M	0.552 ± 0.040	0.486 ± 0.033	0.568 ± 0.015
	CoA	2.19 ± 0.17	2.47 ± 0.35	2.98 ± 0.25
	Total	3.96 ± 0.16	4.05 ± 0.29	4.79 ± 0.25
Kidney	A	2.93 ± 0.27[a]	3.64 ± 0.14[a]	7.24 ± 0.38[b]
	M	0.326 ± 0.017[a]	0.391 ± 0.021[a]	1.02 ± 0.12[b]
	CoA	0.496 ± 0.456	0.486 ± 0.268	<0.025
	Total	3.75 ± 0.58[a]	4.51 ± 0.21[a]	8.27 ± 0.42[b]
Heart	A	6.77 ± 0.55	5.48 ± 0.70	5.50 ± 0.98
	M	3.43 ± 0.47	3.13 ± 0.37	2.21 ± 0.38
	CoA	13.7 ± 2.2	11.0 ± 2.6	11.5 ± 3.0
	Total	23.9 ± 2.4	19.6 ± 3.0	19.2 ± 3.0
Skeletal muscle	A	1.78 ± 0.09[a]	1.76 ± 0.12[a]	0.523 ± 0.185[b]
	M	0.823 ± 0.064[a]	0.789 ± 0.061[a]	1.22 ± 0.14[b]
	CoA	3.37 ± 0.40[a]	2.81 ± 0.40[a,b]	1.56 ± 0.35[b]
	Total	5.97 ± 0.52[a]	5.36 ± 0.56[a]	3.29 ± 0.37[b]
Perirenal adipose tissue	A	0.192 ± 0.067	0.478 ± 0.243	0.067 ± 0.018
	M	0.198 ± 0.080	0.417 ± 0.224	0.188 ± 0.087
	CoA	0.398 ± 0.333	1.80 ± 1.21	0.380 ± 0.315
	Total	0.788 ± 0.472	2.69 ± 1.65	0.635 ± 0.418
Brown adipose tissue	A	13.1 ± 1.4	18.6 ± 3.2	12.6 ± 1.6
	M	6.36 ± 1.07	7.35 ± 1.63	4.21 ± 0.84
	CoA	36.4 ± 6.2	32.1 ± 2.8	39.4 ± 5.3
	Total	55.8 ± 7.2	58.0 ± 6.2	56.2 ± 7.5
Epididymal adipose tissue	A	0.344 ± 0.137	0.125 ± 0.080	0.019 ± 0.006
	M	0.140 ± 0.041	0.058 ± 0.032	0.024 ± 0.010
	CoA	0.609 ± 0.266	0.075 ± 0.052	0.056 ± 0.016
	Total	1.09 ± 0.44	0.258 ± 0.162	0.099 ± 0.011

"A", "M", "CoA", and "Total" indicate acetyl-CoA, malonyl-CoA, CoASH, and total CoA, which is defined here as the sum of the three CoA pools, respectively. All data are expressed as nmol/g of tissue and means ± SEM ($n = 6$).
[a,b]Significance was analyzed by the one-way AVOVA with Tukey's multiple comparison tests. Mean values with different letters were significantly different ($P < 0.05$).

Next, the effects of three different diets on the size and composition of CoA pools in non-brain tissues were evaluated. Larger

total CoA pools were formed in the liver, heart, and brown adipose tissue, and this was not dependent on the nutrient composition of the diets. Significant differences between diets were found only in the liver, not in the heart or brown adipose tissue. The intake of HFD for 4 weeks induced much lower levels of total CoA in the liver, approximately one-fifth of hepatic pool sizes from HCD- and HPD-fed rats. This drastic reduction in the total CoA pool was brought about by decreased CoASH content. Two possibilities are (i) the degradation of CoASH by liver-specific CoA-phosphodiesterase [23], such as is seen in the shift from fasting to feeding states [18,24–26], and (ii) the formation of medium- and/or long-chain acyl-CoAs which are unmeasurable CoA species in the acyl-CoA cycling method. In addition, malonyl-CoA and CoASH levels were significantly suppressed by the intake of a high-fat diet. Lower levels of hepatic malonyl-CoA should facilitate the incorporation of fatty acids into mitochondria. Thus, hepatic metabolism involving CoASH and acyl-CoAs seems to be greatly influenced by the nutrient composition of the diet. In particular, it was noteworthy that CoA content dramatically changed in the liver, where lipid metabolism is active, and long-term fat intake triggered this change. Besides the liver, total CoA pools in the kidney and skeletal muscle from rats fed HFD were significantly higher and lower, respectively, than those from rats fed on HCD and HPD. Perirenal and epididymal adipose tissues showed the lowest CoA contents among the 13 tissues estimated according to our previous analysis of 10-week-old male rats fed on standard laboratory chow [18], and there were no significant differences in the three CoA pools in spite of the significant accumulation of lipids.

The data obtained in this study provide available information for understanding diet-induced obesity. To elucidate the considerable decrease of CoA content and its effect on lipid metabolism in the liver, it is necessary to analyze the expressions and activities of the enzymes related to fatty acid synthesis and β-oxidation as well as CoA biosynthesis.

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